CHANGES IN THE DISTRIBUTION OF NITROGEN DURING METAMORPHOSIS OF THE MEALWORM, TENEBRIO MOLITOR LINNAEUS[1]

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Needham (1929) stated that during insect metamorphosis when larval tissues are histolyzed there should be a breakdown of the insoluble protein with a simultaneous increase in the soluble protein fractions. As the adult tissues are formed this process should be reversed. This hypothesis was verified by Evans (1932) with the sheep blowfly, *Lucilia sericata;* Anderson (1948) with the Japanese beetle *Popillia japonica;* and by Del Vecchio (1955) with the housefly, *Musca domestica.* However, Evans (1934) found no changes in the various nitrogen fractions during the metamorphosis of the mealworm, *Tenebrio molitor.*

Since this insect is also holometabolic, it seemed improbable that there would be no major changes in the nitrogenous fractions during its metamorphosis. The present study is a reinvestigation of the distribution of nitrogen during each day of metamorphosis in the mealworm, *T. molitor* at 30° C.

MATERIAL AND METHODS

Cultures were maintained at room temperature (approximately 25° C.) in chick growing mash. Water was provided by wetting the cloth covers of the cultures weekly. Mature larvae and prepupae were weighed and vacuum desiccated over anhydrous $CaCl_2$. Prepupae were also collected and placed in an incubator maintained at 30° C. Upon pupation the insects were placed in dated beakers and kept at 30° C. In this manner pupae, timed to within 24 hours, were obtained. At the desired stage of metamorphosis, they were weighed and vacuum desiccated. All material was kept in desiccators until ready for use.

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Fractionation was accomplished by the technique of Del Vecchio (1955). Each insect was pulverized and thoroughly mixed with 10 ml. of distilled water. To the supernate were added 1 ml. of $\frac{2}{3}$ N H_2SO_4 and 1 ml. of 10 per cent sodium tungstate to separate fraction B (water soluble protein not precipitated by tungstic acid) from fraction C (water soluble protein precipitated by tungstic acid). The residue remaining after the previous extractions was treated with a solution of ether-alcohol (1 ml. of distilled water, 4.5 ml. of absolute ethyl alcohol and 4.5 ml. of absolute ethyl ether) to remove fraction A (lipid nitrogen) from fraction D (water insoluble nitrogen). These fractions are given letters of designation so as to correspond with similar fractions obtained by Ludwig and Rothstein (1952) and Del Vecchio (1955). The Kjeldahl procedure was employed to make the nitrogen determinations on each fraction.

OBSERVATIONS

No loss in the percentage of nitrogen occurred during the change from larva to adult. However, there was an increase in the total nitrogen percentage of the adult.

The changes in the distribution of nitrogen for each day of metamorphosis are given in table I. Each fraction is expressed

TABLE I

CHANGES IN THE DISTRIBUTION OF NITROGEN DURING THE METAMORPHOSIS OF THE MEALWORM. NITROGEN VALUES ARE GIVEN AS PER CENT TOTAL NITROGEN WITH THEIR STANDARD ERRORS.

	Fraction A	Fraction B	Fraction C	Fraction D
Larva	1.82 ± 0.23	13.41 ± 2.08	17.18 ± 1.75	67.59 ± 1.3
Prepupa	1.92 ± 0.12	11.57 ± 0.80	10.60 ± 0.63	75.96 ± 1.0
Newly molted pupa	2.97 ± 0.19	15.75 ± 0.41	12.85 ± 0.37	68.52 ± 0.52
1-day pupa	2.15 ± 0.13	15.28 ± 0.86	12.06 ± 0.43	70.54 ± 0.74
2-day pupa	2.00 ± 0.11	13.98 ± 0.51	11.33 ± 0.38	72.77 ± 0.87
3-day pupa	1.98 ± 0.11	15.74 ± 0.80	12.31 ± 0.59	70.04 ± 0.91
4-day pupa	1.65 ± 0.12	14.80 ± 0.80	11.66 ± 0.64	72.21 ± 0.90
5-day pupa	1.32 ± 0.33	16.14 ± 0.79	11.36 ± 0.31	70.73 ± 0.77
Newly emerged adult	1.40 ± 0.11	18.64 ± 0.46	11.91 ± 0.68	68.04 ± 0.77

as per cent total nitrogen. Fraction A (lipid nitrogen) remained constant during the larval and prepupal stages and then increased to 2.97 per cent in the newly molted pupa. This increase was followed by a decrease to 2.15 in the 1-day pupa and then a steady decrease to 1.40 per cent in the newly emerged adult. Fraction B decreased from 13.41 in the larva to 11.57 per cent in the prepupa. This decrease was followed by an increase to approximately 15 per cent in the early pupa and it remained at approximately this value until the last day of the pupal stage. Upon emergence it increased to 18.64 per cent. Fraction C decreased from a high of 17.18 per cent in the larval stage to 10.60 in the prepupal stage. This fraction remained between 11 and 13 per cent throughout the remainder of the life cycle. Fraction D was 67.59 per cent in the larva and increased to 75.96 in the prepupa. This increase was followed by a decrease to 68.52 per cent in the newly molted pupa. This fraction then increased to 70.54 per cent in the 1-day pupa and remained at approximately this value throughout the remainder of the pupal stages. Upon emergence of the adult it decreased to 68.04 per cent. These changes are shown graphically in figure 1. Fraction D (insoluble nitrogen) showed a marked decrease in the newly molted pupa and then gradually increased until the 2-day pupa. The graph shows that nitrogen from fraction D is transferred to A, B and C in the newly molted pupa. Reciprocal shifts are shown between fractions D and B. All of these shifts were shown to be statistically significant.

DISCUSSION

The constancy of the nitrogen percentages are in agreement with those of other workers (Evans 1932, for the blowfly, *Lucilia sericata,* Anderson 1948, for the Japanese beetle, *Popillia japonica,* and Del Vecchio 1955, for the housefly, *Musca domestica*). The increase in the percentage of total nitrogen obtained upon emergence of the adult may be associated with the shedding of the cuticle and a loss of water which occurs at this time. The results of the present study on the distribution of nitrogen are in accordance with other work on holometabolous insects. The shifts in nitrogen obtained during the metamorphosis from prepupa to pupa indicate a breakdown of larval protein and an increase in the decomposition products. During the early pupal

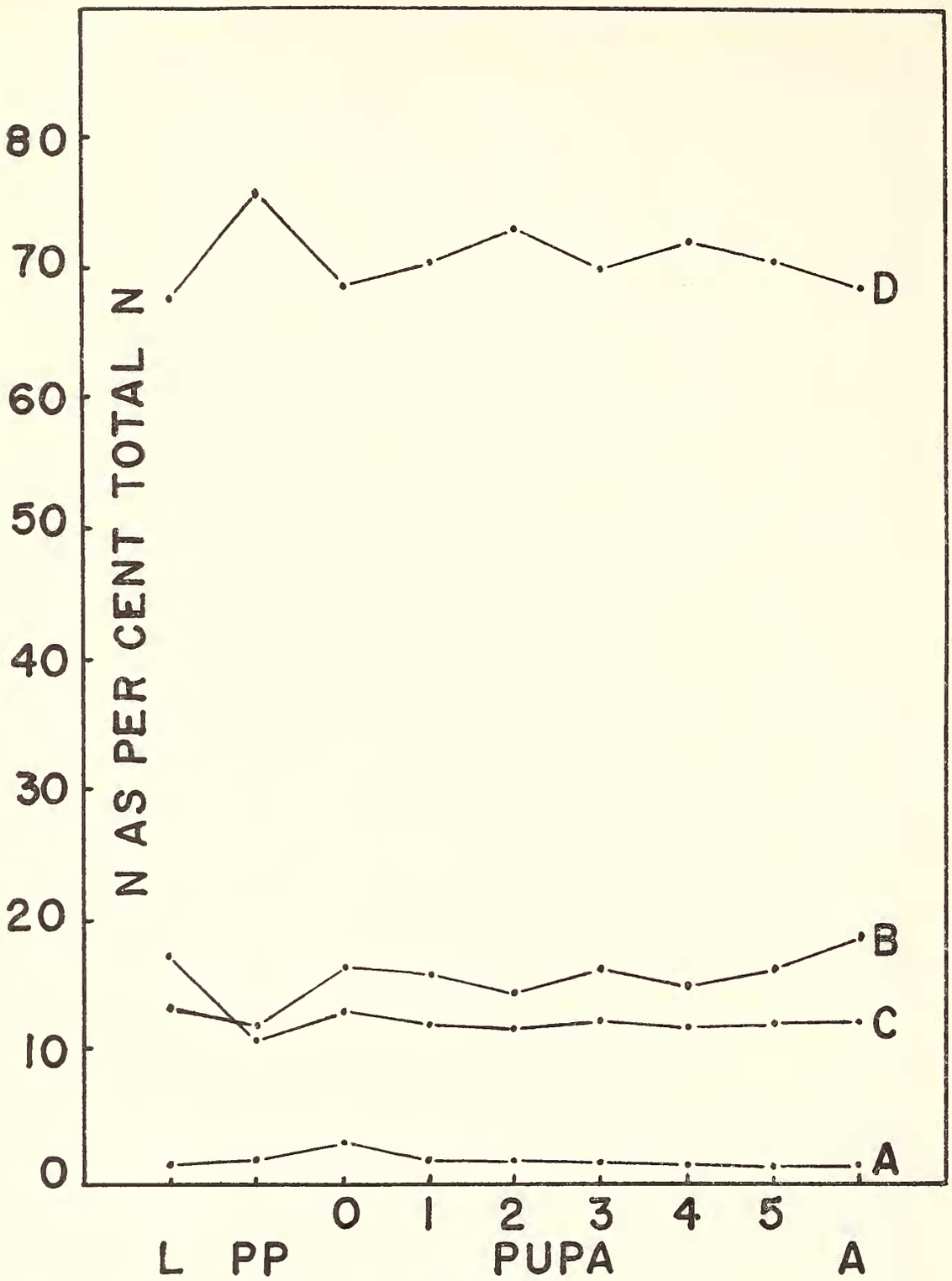

FIG. 1. Changes in the per cent of total nitrogen of the various fractions during the metamorphosis of the mealworm at 30° C. L, larva; PP, prepupa; O, newly molted pupa; 1 through 5 represent days of the pupal stage; A, newly emerged adult.

stages there is an utilization of these products for the synthesis of adult tissues. If the processes of histolysis and histogenesis occur simultaneously during the change from the prepupa to newly molted pupa, the process of histolysis is dominant while

the process of histogenesis is dominant during the early pupal stages. Evans (1934) studied the distribution of nitrogen in the mealworm, *T. molitor* on alternate days during metamorphosis at 25° C. from larva to adult. He obtained the insoluble protein fraction by the addition of distilled water to the powered material. Soluble proteins were precipitated by the addition of trichloroacetic acid to the filtrate, while proteoses and peptones were precipitated with sulphuric acid and sodium tungstate. He failed to show any major shifts and concluded that histolysis and histogenesis are not as clearly defined in Coleoptera as in the higher Diptera. The work of Anderson (1948) on the Japanese beetle has shown the inaccuracy of this generalization since a large decrease in the insoluble and an increase in the soluble nitrogen occurred at pupation. In the present study, complimentary shifts between the nitrogenous fractions were obtained also during the transition from prepupa to pupa but were not as pronounced as those found by Anderson (1948) for the Japanese beetle or by Del Vecchio (1955) for the housefly.

SUMMARY

Nitrogen fractionations were made on the mealworm, *Tenebrio molitor,* collected at 24 hour intervals during metamorphosis at 30° C.

The change from larva to adult showed no loss in the percentage of nitrogen but a slight increase occurred upon the emergence of the adult.

During metamorphosis the insoluble proteins (fraction D) decreased sharply from 76.0 in the prepupa to 68.5 in the newly molted pupa. It then increased to 72.8 per cent in the 2-day pupa and remained at approximately this value during the remainder of the pupal stage. Upon emergence of the adult it decreased to 68.04 per cent. Reciprocal shifts are shown in fraction B. Nitrogen from fraction D was transferred to A, B and C in the newly molted pupa.

The complimentary shifts between the nitrogenous fractions may indicate the breakdown of the larval protein during the transition from prepupa to pupa and the synthesis of adult protein during the remainder of the pupal period. However, these shifts were not as pronounced in this species as in some other holometabolous insects.

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Mr. Farb proposed that Mr. Tony Roberts be appointed to report to the Executive Committee on the possibilities of forming a Junior Entomological Society under the sponsorship of the Society. A substitute motion was made and passed that Miss Alice Gray and Mr. Roberts be invited to the December 3rd meeting of the Executive Committee to report on possibilities of such a move.

Dr. M. J. Ramsey, Training Officer of the Plant Quarantine Division, U. S. Department of Agriculture spoke on "Insects In International Commerce." He reported that 5000 years ago the granary weevil was found in the tombs of the Pharoahs and was probably the first insect transported around the known world in commerce. In the recent book *Faunal Connections Between Europe and North America*, Lindroth delves into the records of early explorers of the New World and concludes that since their ballast was soil, many insects were carried with them. And the second voyage of Columbus might very well have been the means of bringing European insects to this country, since he was carrying plants to propagate in the New World.

It is in cargo shipments that we today find the bulk of insects entering international commerce, said Dr. Ramsey. The mails, too, can be a means of dissemination; for example, USDA recently found the Khapra beetle in rice seeds mailed from Asia. One of plant quarantine's major problems is shamrocks sent from Ireland, since golden nematode cysts are often found in the soil accompanying them. The increase in international air travel increases the problem of quarantine, since serious pests can survive the short flights.

Of our present pests, records kept between 1854 and 1904, before there was a federal quarantine, show that 100 pests of agriculture became established here from abroad. Since then, there have been very few. To show the magnitude of the job in keeping foreign injurious insects from our borders, Dr. Ramsey said that the yearly average for the last decade has been the interception of 6763 shipments that contained pests not yet established in this country.

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